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# Total Synthesis of Kalimantacin A

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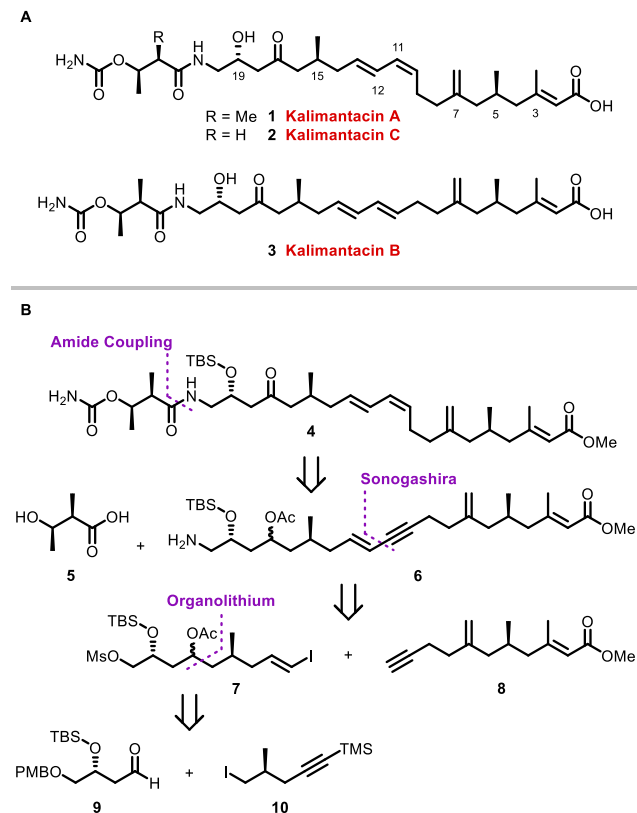
**ABSTRACT:** The kalimantacins are a family of hybrid polyketide-nonribosomal peptide derived natural products that display potent and selective antibiotic activity against multi-drug resistant strains of *Staphylococcus aureus*. Herein, we report the first total synthesis of kalimantacin A, in which three fragments are prepared and then united via Sonogashira and amide couplings. The enantioselective synthetic approach is convergent, unlocking routes to further kalimantacins and analogues for SAR studies and clinical evaluation.

The kalimantacins (**1-3**, Scheme 1A) are a family of bacterial metabolites of mixed polyketide-nonribosomal peptide synthase (PKS-NRPS) origin that display potent antibiotic activity.<sup>1</sup> Originally isolated from a soil sample collected from the West Kalimantan region of Borneo, they are produced by several bacteria including *Pseudomonas fluorescens* and show high selectivity against multi-drug resistant *S. aureus* species including methicillin-resistant *S. aureus* (MRSA).<sup>2,3</sup> The most active and abundant member is kalimantacin A **1** (also known as batumin),<sup>3</sup> whose relative and absolute stereochemistry was determined in 2015 through a combination of degradation studies and fragment synthesis.<sup>4</sup> Preliminary clinical research has indicated that topical application of kalimantacin A is highly effective at disrupting and preventing the formation of *S. aureus* biofilms.<sup>5</sup>

Another fascinating aspect of these natural products is their incorporation of  $\beta$ -branches, a structural feature present in many polyketides with important biological activities.<sup>6</sup> Uniquely, the kalimantacins contain three different and sequential types of  $\beta$ -branches at C-3, C-5 and C-7, as well as an additional methyl group at C-15. The biosynthetic mechanisms that control this remarkable selectivity have recently been investigated.<sup>6</sup>

The anti-staphylococcal activity of the kalimantacins derives from inhibition of FabI, an enoyl-acyl carrier protein reductase enzyme that plays an essential role in fatty acid biosynthesis.<sup>7</sup> X-ray crystallography and molecular dynamics simulations have shown that the kalimantacin binding mode is unique, differing significantly from other FabI inhibitors.<sup>8</sup> The stage is now set to gain further insights into

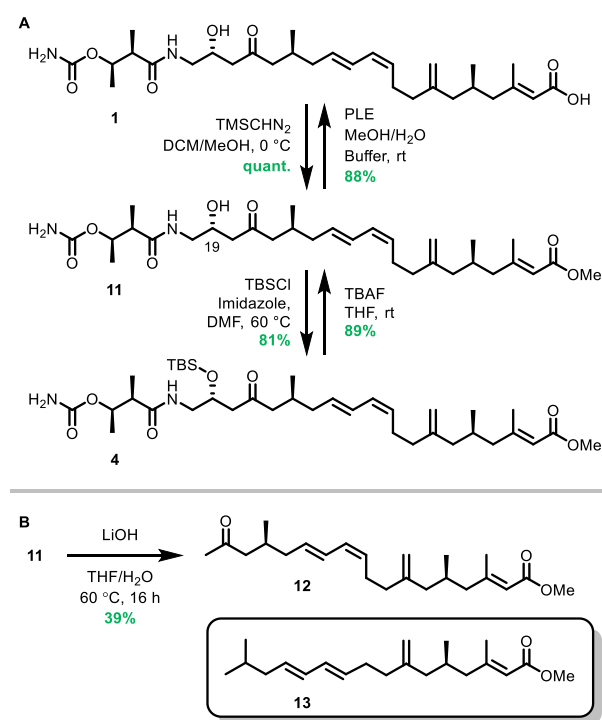
**Scheme 1. (A) Selected Kalimantacin Natural Products. (B) Retrosynthetic Analysis of Kalimantacin Ester 4.**



the structure-activity relationships (SAR) of the kalimantacins, which will inspire future anti-staphylococcal drug development. To realize this potential, access to a library of analogues is now urgently required. Herein, we describe the first total synthesis of kalimantacin A **1** via an enantioselective, flexible and convergent approach.

In our retrosynthetic analysis it was anticipated that kalimantacin A could be prepared from protected ester **4** (Scheme 1B). From here, a late-stage amide coupling of (2*R*,3*R*)-3-hydroxybutanoic acid **5** and amine **6** was proposed. The (*E,Z*)-diene was to be prepared via a Sonogashira coupling of vinyl iodide **7** and alkyne **8**, followed by stereoselective reduction of the enyne. It was envisaged that **7** could be assembled through lithiation of iodide **10** and reaction with aldehyde **9**. This convergent approach was designed to be versatile and readily adaptable in terms of both chain length and functionality to synthesize analogues for SAR studies, as informed by X-ray crystallography and molecular dynamics simulations with FabI. Furthermore, the route was to be exploited for the preparation of putative biosynthetic intermediates to further probe the mechanisms of  $\beta$ -branch incorporation in polyketide biosynthesis.

### Scheme 2. Semi-Synthetic Studies of Kalimantacin A.

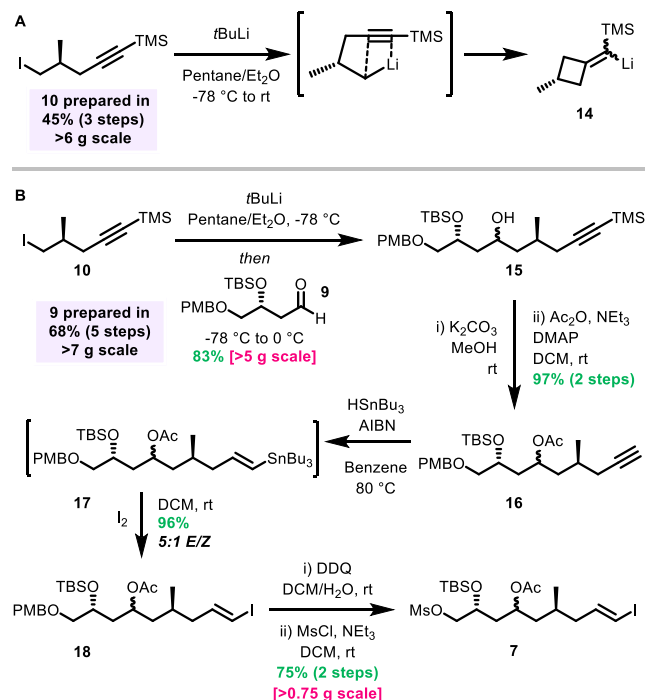


To begin, it was important to establish if the proposed protecting groups of **4** could be readily removed in the final stages of the total synthesis. Thus, kalimantacin A **1** was isolated from cultures of *P. fluorescens* in titers of 65 mg/L using a procedure adapted from Mattheus et al.<sup>3</sup> The acid was methylated with TMSCHN<sub>2</sub> to ester **11** and the 19-hydroxyl was protected as the TBS ether to give **4** in 81% yield over two steps (Scheme 2A). To ensure these protecting groups could be removed cleanly, silyl ether **4** was treated with TBAF giving alcohol **11** in 89% yield. However, treatment of

**11** with LiOH at room temperature returned only starting material and on heating to 60 °C gave methyl ketone **12** via retro-aldol fragmentation of the  $\beta$ -hydroxy ketone moiety (Scheme 2B). This compound is of interest for SAR studies as it closely resembles another natural product, **13**, which was isolated from a fresh-water sponge and also displays activity against Gram-positive bacteria including *S. aureus*.<sup>9</sup> Hydrolysis of methyl ester **11** was achieved cleanly using porcine liver esterase (PLE), giving kalimantacin A **1** in 88% yield.

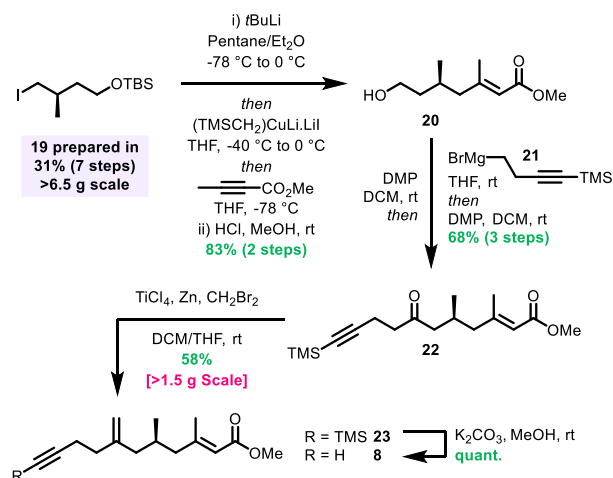
Turning to the total synthesis of kalimantacin A, vinyl iodide **7** (C-12 to C-20) was assembled from two building blocks, aldehyde **9** and alkyl iodide **10**, which were both prepared on a multigram scale using previously reported procedures (see Supporting Information). Aldehyde **9** was synthesized in five steps and 68% overall yield starting from the chiral epoxide (S)-(-)-glycidol.<sup>10</sup> Iodide **10** was prepared in three steps and 45% yield from a commercially available propionylated Evans auxiliary.<sup>11</sup>

### Scheme 3. (A) Lithiation and Rearrangement of Iodide 10. (B) Synthesis of Vinyl Iodide 7 (C-12 to C-20).



Lithiation of iodide **10** using *t*BuLi was required for the coupling with aldehyde **9**. It was found that only poor conversion to **15** was observed at low temperatures, with warming to 0 °C required to obtain full consumption of aldehyde **9**. However, at temperatures above ~-20 °C the alkyl lithium cyclized to give cyclobutylidene **14** (which was trapped by an electrophile), meaning careful temperature control was required (Scheme 3A).<sup>12-14</sup> Additionally, if too large an excess of *t*BuLi was employed (>1.5 equivalents with respect to iodide **10**) then a secondary alcohol was formed via addition to aldehyde **9**. Following optimization, desired alcohol **15** was isolated as a mixture

**Scheme 4. Synthesis of Alkyne 8 (C-1 to C-11).**

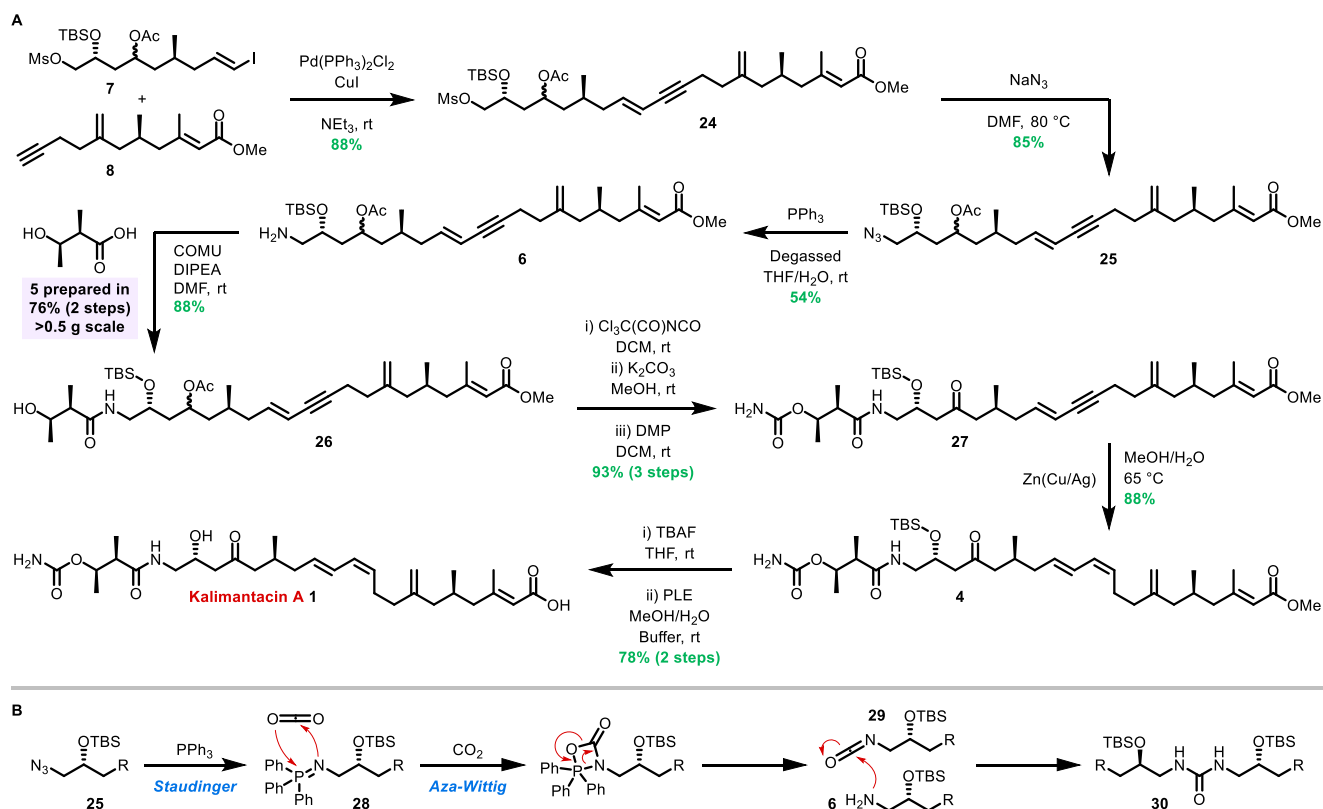


of diastereomers in 83% yield and the coupling was successful on a multigram scale (Scheme 3B). Removal of the TMS group, using  $K_2CO_3$  in MeOH followed by acetylation gave ester **16**. The (*E*)-vinyl iodide was installed through hydrostannation of the alkyne to give **17**, followed by *in situ* treatment with iodine to give **18**. Deprotection was undertaken at this stage to give the corresponding alcohol, as DDQ was found to give a complex mixture post-Sonogashira coupling. Finally, the alcohol was transformed to mesylate **7** in 75% yield over two steps.

Preparation of alkyne **8** (C-1 to C-11) was based upon our previous work used in the structural assignment of kalimantacin A in which iodide **19** was used as a synthetic intermediate (Scheme 4).<sup>4</sup> **19** was subjected to lithium-halogen exchange with *t*BuLi, then added to a freshly prepared solution of trimethylsilylmethylcopper to generate a mixed cuprate species in which the trimethylsilylmethyl group acts as an activating but non-transferable ligand.<sup>15,16</sup> Conjugate addition to methyl but-2-ynoate proceeded with complete stereocontrol for the (*E*)-alkene then the TBS group was cleaved under acidic conditions to liberate alcohol **20** in 83% yield over two steps. Oxidation was undertaken using Dess-Martin periodinane (DMP) to provide the aldehyde, which was treated with Grignard reagent **21**, formed from the corresponding bromide.<sup>17</sup> The resulting mixture of alcohols was again oxidized using DMP to give ketone **22** as a single enantiomer in 68% yield over three steps. The next stage was the introduction of the *exo*-methylene moiety which had been undertaken previously using Tour methylenation conditions (Cp<sub>2</sub>ZrCl<sub>2</sub>, CH<sub>2</sub>I<sub>2</sub>, Zn).<sup>4,18</sup> While this was successful, the reaction was capricious giving variable yields. Instead, it was found that a Lombardo methylenation (TiCl<sub>4</sub>, CH<sub>2</sub>Br<sub>2</sub>, Zn)<sup>19</sup> was more robust and reproducible, providing **23** in 58% yield on a gram scale. Finally, deprotection was undertaken using K<sub>2</sub>CO<sub>3</sub> in MeOH to give alkyne **8**.

The Sonogashira coupling of vinyl iodide **7** and alkyne **8** proceeded smoothly to give enyne **24** in 88% yield, thus

**Scheme 5. (A) Completing the Total Synthesis of Kalimantacin A. (B) Formation of Urea 30.**



completing the construction of the C-1 to C-20 carbon backbone of kalimantacin A **1** (Scheme 5A). The next step of the synthesis required conversion of mesylate **24** to azide **25**. Maeda and co-workers had reported that the steric bulk of a TBS group hindered nucleophilic substitutions in similarly protected 1,2-diols.<sup>20</sup> However, heating mesylate **24** with NaN<sub>3</sub> gave azide **25** in 85% yield. Reduction of azide **25** to amine **6** was achieved using a Staudinger reaction employing PPh<sub>3</sub> in a mixture of THF and H<sub>2</sub>O. This reaction required very careful degassing, as in the presence of air, rather than the required hydrolysis of the intermediate iminophosphorane **28**, an aza-Wittig reaction with carbon dioxide occurred to give isocyanate **29**, which further reacted to give dimeric urea species **30** (Scheme 5B).<sup>21,22</sup>

The final fragment, acid **5**, was prepared in two steps and 76% yield from ethyl (*R*)-3-hydroxybutanoate (see Supporting Information). Coupling of hydroxy acid **5** and amine **6** was undertaken with no protection of the alcohol, thus obviating the need for further protecting group manipulations. Using COMU and *i*Pr<sub>2</sub>NEt, hydroxy-amide **26** was isolated in 88% yield. Carbamoylation of **26** using trichloroacetyl isocyanate, was followed by concomitant hydrolysis of both acetates with K<sub>2</sub>CO<sub>3</sub> in MeOH. Oxidation of the resultant alcohol with DMP provided ketone **27** in 93% yield over three steps.

The final key step of the total synthesis was the selective reduction of the (*E*)-enynone **27** to generate the required (*E,Z*)-diene **4**. In this case, commonly used hydrogenation with a poisoned palladium catalyst was avoided due to concerns of achieving the required chemoselectivity. Instead, **27** was treated with zinc activated with copper and silver, which gave the desired (*E,Z*)-diene **4** in 88% yield as a single isomer by <sup>1</sup>H NMR spectroscopy (*J* = 15 Hz and 11 Hz respectively).<sup>23</sup> The optical rotation of diene **4** prepared by total synthesis was in excellent agreement with the reference material obtained via protection of kalimantacin A **1** isolated from cultures of *P. fluorescens* (Scheme 2). Finally, following the previously established deprotection and ester hydrolysis protocols (Scheme 2), completed the first total synthesis of kalimantacin A **1**. The NMR spectral data and HPLC retention time were in accord with an authentic sample of the natural product (see Supporting Information).

In conclusion, herein we have reported the first total synthesis of the PKS-NRPS derived antibiotic natural product kalimantacin A **1**. The enantioselective and convergent approach unites three fragments via Sonogashira and amide couplings. This flexible synthetic strategy, combined with recent insights into kalimantacin binding to FabI<sup>8</sup> will enable SAR studies to be undertaken, thus paving the way for the design of future selective anti-staphylococcal drugs and their development for clinical assessment. Additionally, this route will be adapted for the synthesis of putative biosynthetic intermediates to further probe the mechanisms of  $\beta$ -branch incorporation in kalimantacin biosynthesis.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at <http://pubs.acs.org>.

Experimental procedures and spectral data for all new compounds (PDF).

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### Notes

The authors declare no competing financial interest.

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